BACTERIAL LUMINESCENCE IN THE DEEP-SEA ANGLERFISH *ONEIRODES ACANTHIAS* (GILBERT, 1915)[1]

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ABSTRACT: The luminous organ (esca) of the deep-sea anglerfish *Oneirodes acanthias* (family Oneirodidae, suborder Ceratioidei, order Lophiiformes) has been studied by means of light and electron microscopical methods. The escal lumen contains numerous rodlike bacteria which are Gram-negative and without spores, capsules, or flagella. Ultrastructural features of the bacteria include a double-layered cell wall and mesosomal invaginations of the plasma membrane. Although the microorganisms grow on seawater nutrient broth or agar, no luminescence has been observed under these conditions.

INTRODUCTION

Adult female ceratioid anglerfishes, except *Caulophryne* and *Neoceratias,* bear a luminous organ called the esca at the distal tip of a modified first dorsal ray (Figs. 1 and 2). The name of the luminous organ, which means bait in Latin, evokes speculation about its use as a lure. Whether it attracts prey by mimicking some small luminous animal or whether it signals conspecific males remains an elusive problem; it might even serve both purposes in the life history of some anglerfishes (Bertelsen, 1951; Marshall, 1971; Pietsch, 1972).

That luminescence in anglerfishes involves symbiotic bacteria has been suspected since Dahlgren's (1928) elementary description of the esca in *Ceratias.* Nevertheless, Brauer (1908) concluded that only secretory granules were present and recently Haneda (1968) doubted that the luminescence of *Himantolophus groenlandicus* was bacterial, after he had observed the light and failed to culture microorganisms from the organ. Bassot (1966) stated that bacteria are present in the escas of *Linophryne* and *Gigantactis* [erroneously referred to as *Mancalias* in Bassot (1966) according to Hulet and Musil (1968)] on the basis of his own unpublished electron micrographs. Bassot (1966) also saw bacteria in histological sections of the escas of *Ceratias* and *Melanocetus.* The electron microscopic study of Hulet and Musil (1968)

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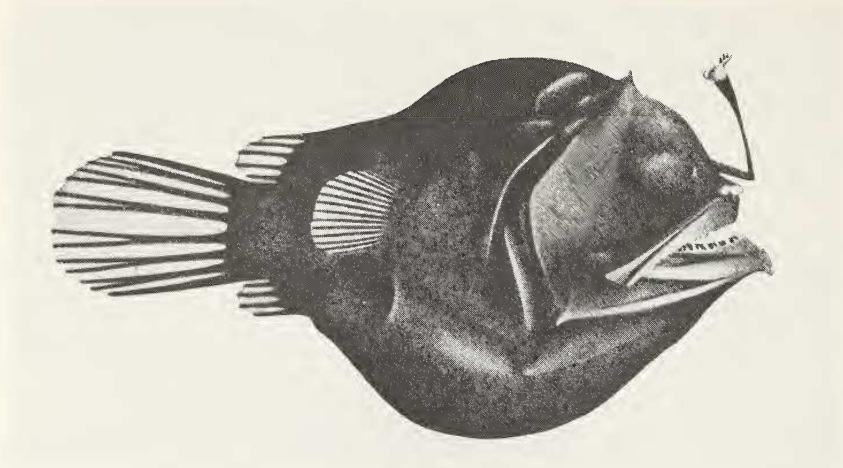

FIGURE 1. *Oneirodes acanthias*. From the original description by Gilbert (1915).

showed that there are bacteria in the esca of *Melanocetus murrayi* but the fixation limited the quality of ultrastructural detail. The results presented below confirm that bacteria are present in the esca of *Oneirodes acanthias* through bacteriological staining methods, light and electron microscopy, and growth of the microorganisms on artificial media.

MATERIALS AND METHODS

Fishes were collected in deep waters off southern California. Immediately after capture they were placed in a basin of cooled seawater and taken to the darkroom where their luminescence was observed. No emission spectra were recorded but the color appeared blue or blue-green to my eyes.

Electron Microscopy

Tissue from live specimens was fixed in 2.5% glutaraldehyde in phosphate buffer with added sodium chloride, pH 7.3-7.4, according to either Karlsson and Schultz (1965) or Millonig (1961) for one to one and one-half hours, rinsed in buffer, then postfixed in 1% osmium tetroxide in either of the two buffers just mentioned. Fixation was usually done at room temperature. Specimens were stored temporarily in the cold (about 5°C) on board ship, either in buffer or after being brought to 70% ethanol through a graded series of ethanol-water mixtures. After returning to the shore lab, dehydration was completed and the specimens were embedded in epoxy resin. Thin sections (500-1000 A) were cut with a diamond knife on an MT-2 or LKB ultramicrotome. These sections were picked up on naked

FIGURE 2. Enlarged view of the esca of a specimen of *O. acanthias*. The escal bulb is partly covered with heavily pigmented skin of the illicium. A long, partially pigmented appendage extends anteriorly; a smaller unpigmented appendage is posterior. The scale equals one mm.

300-mesh grids and then stained in freshly prepared uranyl acetate (1-2% in 50% ethanol, filtered through a millipore before use) for thirty minutes at 40°C, followed by Reynold's lead citrate, also freshly prepared, for five to ten minutes. They were examined and photographed in an RCA EMU-3F electron microscope.

Light Microscopy

Sections one micron thick were cut from blocks prepared in the above manner and stained briefly in 0.1% toluidine blue in 1% borax. One esca, obtained from a museum specimen originally fixed in 10% formalin in seawater and preserved in 40% isopropanol, was embedded in paraffin for serial sectioning. Another esca was embedded in glycol methacrylate (Feder and O'Brien, 1968). Paraffin and glycol methacrylate sections were stained with acid fuchsin and toluidine blue.

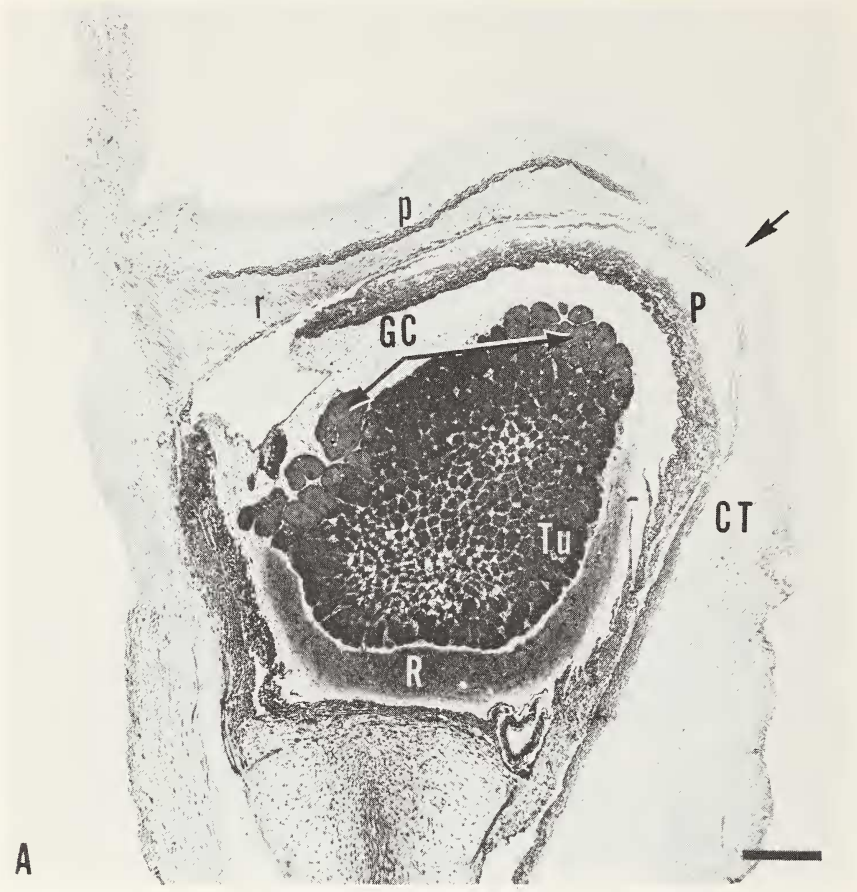

FIGURE 3A. (Above) Longitudinal section of the esca. This section does not pass through the pore which leads to the seawater but its location is indicated by an arrow on the posterior surface of the esca. B (Opposite page) The same section viewed through crossed polarizers, showing the birefringent crystalline reflectors. The scale for both figures equals 200 microns. Abbreviations: CT, connective tissue; GC, large gland cells; P, main pigment layer; p, accessory pigment layer; R, main reflector layer; r, accessory reflector layer; Tu, tubules lined with glandular epithelium and containing numerous bacteria.

Bacteriology

Escal smears were prepared from live anglerfishes. The esca was rinsed briefly in 100% ethanol, then either pressed on a clean glass slide to extrude some luminal matter through the escal pore, or cut with a sterile razor blade to expose the lumen, the contents of which were then streaked on clean glass

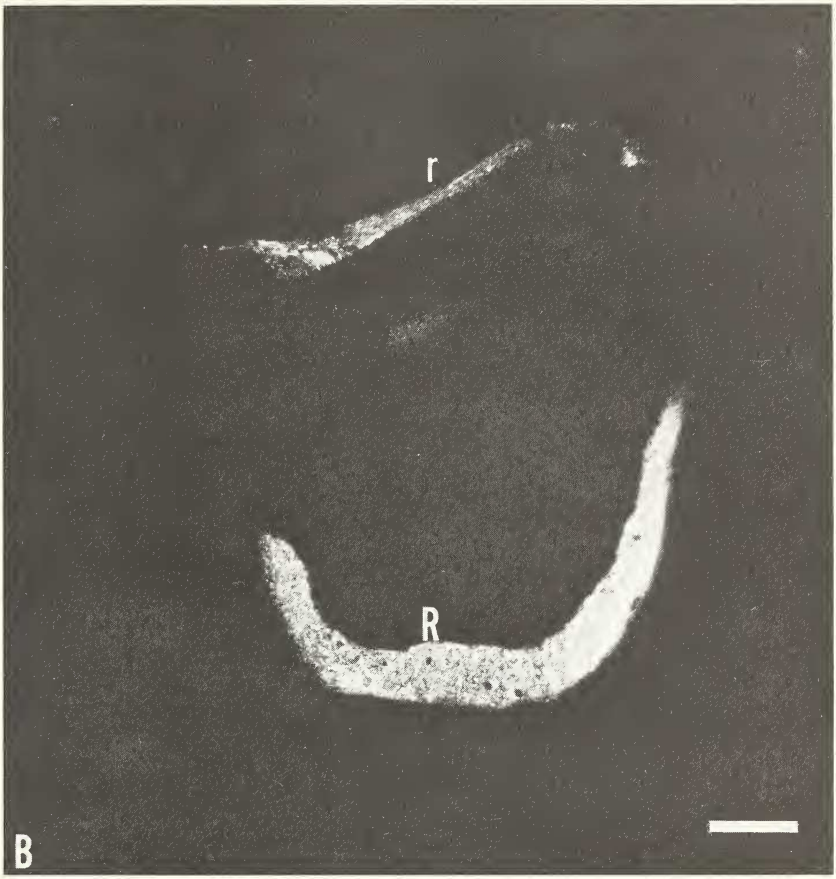

FIGURE 3B.

slides. The smears were dried on a warm hotplate. They were stained and examined right away or later in the shore lab, following a few days storage in the cold. The stains employed were the Gram stain (Lillie, 1965), Fontana's silver stain for flagella, Malachite green for spores, and Muir's stain for capsules (the latter three stains as described by Baker, 1967).

Whole bacteria were also examined by electron microscopy after drops of escal exudate had been allowed to evaporate on grids coated with collodion and stabilized with carbon. The specimens were then shadowed with platinum-palladium before examination.

To prepare a culture of the bacteria from the esca, the organ was first rinsed with sterile distilled water, then dipped in 70% ethanol for about ten

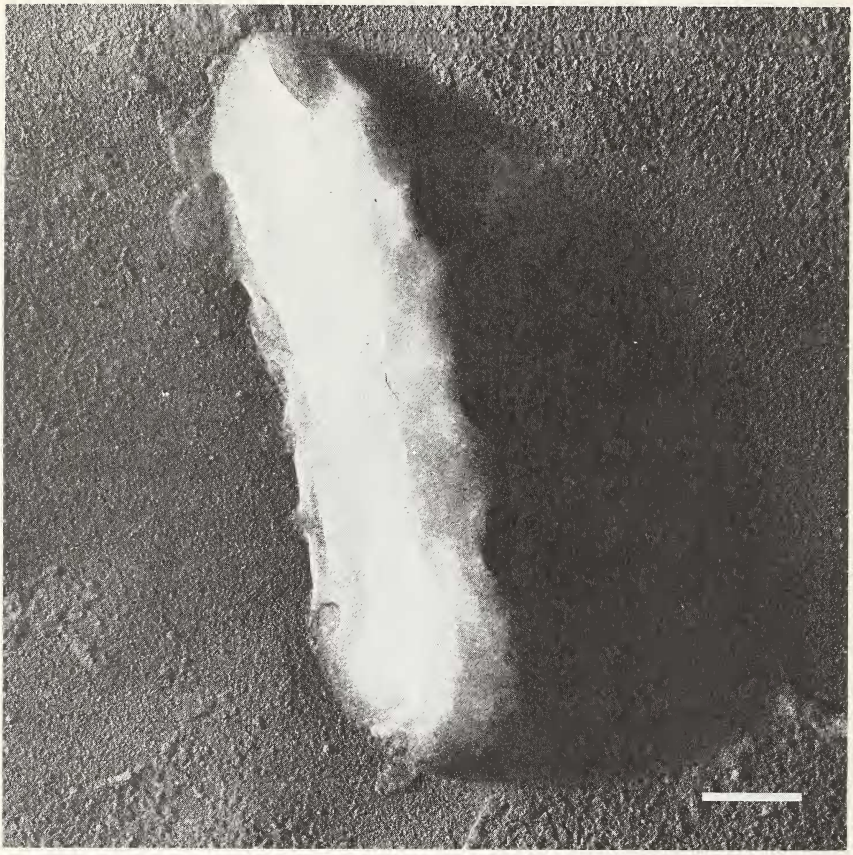

FIGURE 4. Electron micrograph of a whole bacterium from the esca of *O. acanthias,* shadowed with platinum-palladium. The scale equals one micron.

seconds. It was transferred to a sterile watchglass containing 0.5 ml sterile nutrient broth. The esca was pressed gently with a sterile pipette to extrude some material from the lumen. The innoculated nutrient broth was pipetted into several test tubes of sterile broth. Control tubes, which were not innoculated but were otherwise exposed to the same conditions as the innoculated ones, remained free of contamination. The culture was grown at room temperature. The medium used (Hastings and Mitchell, 1971) contained 8 g of nutrient broth (Difco) and 3 ml of glycerol per liter of seawater; it was sterilized by autoclaving.

FIGURE 5. Electron micrograph of a longitudinally sectioned bacterium in the escal lumen. CW, cell wall; Mes, mesosome; PM, plasma membrane. The scale equals 0.5 micron.

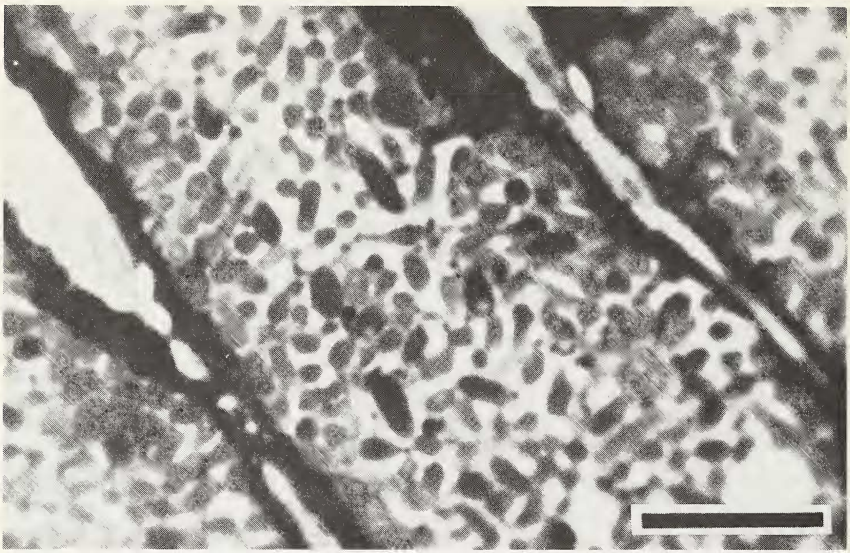

FIGURE 6. Light micrograph of portions of three tubules containing numerous bacteria. The scale equals 10 microns.

Specimens

Eight specimens of *O. acanthias,* 21 to 118 mm in standard length (SL), were used. Observations of bioluminescence were made on seven of them; four were used for light and electron microscopy. The bacterial culture was isolated from a single specimen, 30 mm SL.

RESULTS AND DISCUSSION

Histology

The esca (Fig. 3) consists of the following arrangement of tissues: (1) a very thin external epithelium; (2) a thick layer of connective tissue which gives the esca its characteristic shape; (3) a layer of black pigment surrounding the escal bulb; (4) the main reflector, a cup-shaped layer of oriented crystals just internal to the layer of black pigment; (5) a smaller accessory reflector bordered distally by black pigment near the dorsal surface of the esca; (6) glandular cells, most of which line tubules which are continuous with the lumen of the escal bulb; (7) vascular supply; and (8) a central lumen containing numerous bacteria and communicating with the external environment by means of a pore. Although nerve branches occur in the peripheral connective tissue of the esca, they do not penetrate into the area where the glandular cells and bacteria are.

Staining Properties

Smears from the esca of *O. acanthias,* dyed with selective stains for light microscopy, reveal numerous Gram-negative rods of varying length, some in the process of dividing, which lack capsules, spores, and flagella.

Fine Structure

Studied with the electron microscope, whole bacteria shadowed with heavy metal appear to be aflagellate rods (Fig. 4), as expected from previous light microscopy. Thin sections reveal that the bacteria have a two-layered cell wall just external to the plasma membrane. Internally, ribosomes and a finely filamentous material which probably represents portions of the bacterial genome are present. A fairly common feature of bacterial structure in *O. acanthias,* a mesosome, is evident in Figure 5. The functional significance of these multiple infoldings of the plasma membrane remains uncertain, but it is possible that mesosomes increase the surface area of the cell membrane in order to facilitate such processes as cell division and spore formation and perhaps to provide additional sites for enzymatic attachment (Ryter, 1969; Bisset, 1970).

Many of the bacteria lie in the central lumen, together with a mass of irregularly shaped membranous bodies, most of which are unrecognizable but a few of which suggest degenerating mitochondria. Vesicles of varying shape can also be seen in the lumen. The membranous fragments and vesicles seem to be derived from apocrine secretion by the gland cells. Many other bacteria appear in the tubules lined with glandular epithelium (Fig. 6) or within deep invaginations into the glandular cells.

Vacuolar inclusions, apparently not enclosed by a membrane, occur in some of the bacteria. Similar structures were noted in the bacteria from *Melanocetus* by Hulet and Musil (1968) and they are evident in the electron micrographs by Haneda and Tsuji (1971) of symbiotic bacteria from the luminescent organs of *Photoblepharon* and *Anomalops.* They may represent dilute fluid or unsaturated lipid that was subsequently extracted from the specimen during processing.

Culture of the Bacteria

The conclusive demonstration that bacteria are the source of luminescence requires that they be grown in a pure culture which luminesces. Although I have cultured bacteria from the esca of *O. acanthias,* I have not seen them luminesce. The moneran symbionts in the luminous fishes *Anomalops* and *Photoblepharon* reportedly grow on similar media as non-luminous forms only (Harvey, 1921, 1922, 1952; Haneda, 1943, as cited by Harvey, 1952). In their recent experiments on these fishes, Haneda and Tsuji (1971) obtained only a few colonies, none of them luminescent (Tsuji, personal communication).

Cultures of bacteria from certain other luminous fishes, on the other

hand, emit light readily. Such cultures have been reported from *Malacocephalus laevis* by Haneda (1938); *Physiculus japonicus* by Kishitani (1930); *Monocentris japonica* by Okada (1926); *Acropoma japonica* by Yasaki and Haneda (1936) and Haneda (1950); *Siphamia* spp. by Yoshiba and Haneda (1967) and Haneda (1965); *Cleidopus gloria-maris* by Yoshiba and Haneda (1967); and various species of Leiognathidae by Haneda (1940, 1950) and Hastings and Mitchell (1971).

Perhaps the symbionts in *Oneirodes, Anomalops,* and *Photoblepharon* have lost the capacity to synthesize everything that the chemistry of their luminescence requires, so that they now depend on their hosts to complement their deficiencies. The fine structure of the esca in *O. acanthias* suggests that the bacteria obtain certain nutrients from the tissues of the host, with the possible exception of some dissolved salts and trace elements that might be extracted from seawater. The symbionts probably subsist on the secretory products of the glandular cells and the associated masses of degenerate membranous fragments which float freely in the lumen of the esca.

The existence of a pore leading to the seawater outside the esca satisfies several conditions for the successful long-term culture of any strain of microorganisms. It ensures that neither the tonicity nor the pH of the culture medium will vary and it allows continual removal of dead bacteria and cellular waste as newly formed bacteria and freshly synthesized cellular products take their place. Temperature hardly changes over hundreds of meters in the bathypelagic zone and at a particular depth it remains the same day after day, year after year. The bacteria, then, can depend on the constancy of the microenvironment inside the esca.

Summary

The escal light organ of the deep-sea anglerfish *O. acanthias* contains numerous bacterial symbionts. These microorganisms are Gram-negative rods without capsules, spores, or flagella. Their ultrastructural features include a double-layered cell wall and mesosomes. Since the bacteria grow but do not luminesce in seawater nutrient broth, it appears that the host provides certain nutrients required for luminescence.

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